## REMARKS

The Office Action of November 15, 2001 presents the examination of claims 1-28. Claims 1, 3-4, 6-13, and 15-28 are amended. Claims 29-31 are added. No new matter is inserted into the application.

***Drawings***

The Examiner objects to the drawings. Applicants note the objection and will submit corrected drawings upon the finding of allowable subject matter in the present application.

***Oath/Declaration***

The Examiner requires the submission of a new oath or declaration that acknowledges the filing of foreign applications. Applicants are currently in the process of obtaining the oath and will submit it to the USPTO in due time.

***Rejection under 35 U.S.C. § 112, first paragraph***

*Enablement*

The Examiner rejects claims 1-28 under 35 U.S.C. § 112, first paragraph for an alleged lack of enablement. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner asserts that the specification, while being enabling of insulator sequences that *do not* comprise enhancers or other types of transcriptional regulatory elements, does not provide enablement for insulator sequences that *do* comprise enhancers or other types of transcriptional regulatory elements. As such, the Examiner rejects claims 1-28.

From the rejection, it seems that the Examiner has assumed that 'any DNA sequence' can function or be used as an 'insulator sequence'. It is possible that the Examiner has not found an appropriate definition of the insulator sequence in the claims and therefore, raised this rejection.

However, as described on pages 10 and 11 of the specification, the insulator sequence selected in the present invention is derived from the genomic DNA of a plant. It does not produce any functional RNA or protein when used in the present invention as a part of the insulator sequence. When the insulator sequence is placed between two transcriptional units, one unit containing a lethal gene under the control of a tissue-specific promoter and another containing the selectable marker gene under control of a strong constitutive promoter, then the commonly observed leaky expression of the lethal gene is prevented.

The insulator sequence chosen in the present invention is such that it does not carry any enhancer or regulatory elements. If sequences carrying such enhancers or regulatory

elements are used, then the insulator may affect expression of the neighboring genes. Therefore, contrary to the Examiner's remarks, the present invention is not directed to insulator sequences that *do* comprise enhancers or other types of transcriptional regulatory elements, and as such, no enablement therefor is necessary.

As stated above, the purpose of using the insulator sequence is to negate the deleterious effects resulting from leaky expression of the lethal gene, which is why the insulator should not contain any regulatory elements.

The specific sequences used as "insulator sequences" in the present invention are *topoisomerase* gene of pea and *acetolactate synthase* gene of *Arabidopsis*. The expression "insulator" has been used for the first time by the Applicant for these genes. To describe the insulator in terms of the aforementioned properties, Applicants amend claim 11 and submit new claims 30 and 31. Support for these claims can be found on pages 10 and 11 of the specification.

The Examiner states that the claims do not provide guidance for the insulator sequence *per se*. As state above, the insulator sequence used in the present invention is constructed using *topoisomerase* gene from pea and *acetolactate synthase* gene from *Arabidopsis*. The insulator sequence has already been well described in the specification, especially on pages 9 and 10. The method for the construction of the insulator sequence has also

been set out in example 1 of the specification. A person having average skill in the art will not have any difficulty in identifying such a sequence.

In fact, the components of the insulator construct are conventional and they can be readily obtained from any gene database, such as, www.ncbi.nlm.nih.gov/Entrez/. The coding sequences of various elements of the construct have been published in databases such as:

Pea *topoisomerase I* at Accession Nos. Y14558 and *Arabidopsis acetolactate synthase* at Accession No. X51514 in Entrez Database.

These have also been described as well as in the following publications: Reddy et al, 1998, Plant Mol. Biol. 37:773-784. and Mazur et al, 1987, Plant Physiol. 85:1110-1117. Copies of these publications and print outs of the coding sequences from the gene databases are attached hereto for the Examiner's consideration.

Applicants respectfully submit that the insulator sequence can be obtained from gene Databases. The novelty of the invention resides in developing a novel insulator construct containing various (known) elements in order to prevent leaky expression of a lethal gene in transformed plant tissues.

The Examiner states that all insulator sequences will not prevent undesired transcription of a lethal gene construct, and gives the example of the Cucumis *reductase* gene. The kind of sequence that can be used as an insulator has been described on paragraphs 4 and 5 on page 10 and paragraphs 1, 2 and 3 on page

11 of the application. As state above, this insulator sequence does not produce any functional RNA or protein and does not contain any regulatory elements which would influence expression of any of the neighboring genes. Its sole function is to prevent leaky expression of the lethal gene.

The Examiner's argument of using *reductase* gene fails since the 5' flanking region of the gene suggested by the Examiner generally contains regulatory elements and the 3' flanking region occasionally contains regulatory elements. Such a DNA sequence containing regulatory or enhancer elements cannot be used as an insulator in the construct of the present invention.

The Examiner argues that lethal genes whose transcription is targeted to tissues like the root are not likely to produce living male-sterile plants and therefore constructs in which the lethal gene is linked to any tissue-specific promoter cannot be used to produce male-sterile plants.

The argument of the Examiner is not tenable. There may be lethal genes expressing themselves in tissues other than the tapetum. The invention is not concerned with such genes. The only lethal genes considered in the present invention are those that are expressed in the tapetum, or whose expressional effects are observed in the tapetum. It is for this reason that a tapetum specific promoter is used to control the expression of the lethal gene.

For all of the above reasons, Applicants respectfully submit that the instant claims are enabled by the specification. Withdrawal of the instant rejection is therefore respectfully requested.

*Written Description*

The Examiner also rejects claims 1-28 under 35 U.S.C. § 112, first paragraph, for allegedly not being described in the specification. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner believes that the insulator sequence and the other sequences of the plasmid are not described. The only described sequence is the one of the plasmid in Fig. 1. The Examiner also believes that the invention employs novel plasmids contained in microorganisms.

The Examiner's contentions are not correct. The invention does not employ any new microorganisms or any naturally occurring plasmid contained therein. The invention provides a plasmid which contains known elements as described below and is constructed using conventional techniques well-known to a person skilled in the art. The plasmid and its construction is described on pages 18 and 19 of the application.

What is shown in Fig. 1 is a schematic representation of the insulator construct. The insulator construct contains the

following:

(i) a first transcriptional unit consisting of:

(a) a lethal gene: *barnase* gene

(b) a tissue specific promoter: TA29

(ii) a second transcriptional unit consisting of:

(a) a marker gene: *bar* gene,

(b) a constitutive promoter - CaMV35S,

(iii) an insulator sequence which is composed of the *topoisomerase* gene and the *acetolactate synthase* gene.

The vector used for transformation is a disarmed Ti-plasmid vector pPZP200 as described in Hajdukiewicz et al, 1994, Plant Mol. Biol., 25 989-994.

The sequences of the *bar* and *barnase* genes can be found in Accession Nos. X05822 *(bar)* and M14442 *(barnase)* and in the references Thompson et al, 1987, EMBO J. 6:2519-2523 *(bar)* and Paddon and Hartley,1986, Gene, 40:231-239 *(barnase)*.

The sequences of CaMV35S and TA29 promoters can be found in Accession Nos. X05868 and X52283 respectively and also in the following publications: Fang et al, 1989, Plant Cell, 1:141-150 and Seurinck et al, 1990, Nucleic Acids Res.,18:3403.

The sequences of the *topoisomerase* and *acetolactate synthase* genes can be found in Y14558 (pea *topoisomerase* I) and AY042819 and X51514 *(Arabidopsis acetolactate synthase)* and also in the following publications: Reddy et al, 1998,

Plant Mol. Biol. 37:773-784 (*topoisomerase* I) and Mazur et al, 1987, Plant Physicl. 85:1110-1117 (*acetolactate synthase*).

Thus, the insulator DNA and the vector as well as each component thereof are well published and known to any person skilled in the art.

Since the components of the insulator construct and the plasmid are publicly available, and the method of making the construct is also provided in the description, a skilled person would be able to make the construct without any difficulty.

We also notice that many of the citations referred by the Examiner do not provide the coding sequences.

In the light of the above, to us it appears that no deposit of the plasmid as suggested by the Examiner is required.

For all of the above reasons, Applicants respectfully submit that the instant claims are adequately described in the specification. Withdrawal of the instant rejection is therefore respectfully requested.

***Rejection under 35 U.S.C. § 112, second paragraph***

The Examiner rejects claims 1-28 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Applicants have amended the claims and respectfully submit that the claims, as amended, overcome the rejections brought up by the Examiner. Withdrawal of the rejection under 35 U.S.C. § 112, second paragraph is therefore respectfully requested.

***Rejection under 35 U.S.C. § 102***

The Examiner rejects claims 1-4, 7-10, 13-14, 16-17, 19-20, 24, and 26-28 under 35 U.S.C. § 102(e) for allegedly being anticipated by Williams '433 (USP 5,977,433). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Williams '433 primarily describes development of homozygous male sterile plants and their corresponding maintainer lines. The problem that they identify is the removal of a substantial number of plants from the field during maintenance of male-sterile plants by crossing them with normal pollen producing parent plants. In any given field, the removal of male-fertile plants effectively reduces the potential yield of hybrid seed or the potential yield of male-sterile plants during each round of multiplication for producing plant seed. This is economically unattractive for many important crop species such as corn and oil-seed rape. In order to minimize the number of male-fertile plants which have to be removed, male-fertile maintainer plants have been sought which, when crossed with a male-sterile parent plant, produce

a minimum, preferably nc, male-fertile cffspring. (See column 3, lines 1-20 of Williams '433).

Williams '433 proposes a three-pronged strategy to achieve this objective by using a maintainer gene *(barstar)*, a male sterility gene *(barnase)* and a restorer gene *(barstar)* and developing transgenic lines containing the above three components independently. Two different male sterility promoters (ZM13 and TA29) and two different marker genes *(bar* and nptII) are used in this strategy. In this study, restored plants which are generated by crossing male sterile lines with restorer lines are further crossed with lines containing the maintainer gene. Subsequent selfing of selected progeny over two successive generations generates homozygous male sterile plants and their corresponding maintainer lines.

Thus, the objective sought to be achieved by Williams '433 and the strategy proposed to achieve the same are completely different when compared to the invention of the present application. As such, there is no correlation between Williams '433 and the present invention.

On page 12, the Examiner argues that Williams '433 at column 14, line 40 to column 15 line 49 discloses an "insulator sequence", and cites sequences ID Nos. 2, 3 and 17 as examples. This again is not true, since in this section, Williams '433 describes the construction of plant transformation vectors. The 1200 nucleotide fragment referred

to is a TA29 promoter-barstar-3' end cassette which contains a complete transcription unit encoding the specific inhibitor (barstar) of the ribonuclease, barnase.

This sequence cannot be compared to the insulator described in the present invention because: (1) This sequence produces a functional protein (barstar) whereas the primary criterion for a sequence to qualify as 'insulator' according to the present invention is that it should not produce any functional RNA or protein; (2) It also does not fulfill the length criterion: the sequence is only 1200 nucleotides long (i.e 1.2Kb long) whereas the minimum length of the insulator sequence as outlined in the present invention is 2 kb. This prior art sequence cannot be used to prevent leaky expression since it is too short.

pMDB13 referred in Williams '433 is an intermediary cloning vector which contains the pollen-specific Zm13 promoter from maize. Sequence ID 2 is a plasmid derived from pUC18 and contains transcription units for the npt and barstar genes.

The invention of Williams '433 also does not identify or attempt to solve the problem of leaky expression of lethal genes. In contrast, the invention of the present application addresses all such situations where leaky expression of the lethal gene is observed and is to be prevented. Prior knowledge of any inhibitor protein or any other regulatory

component of the lethal gene is not required to combat leaky expression of the lethal gene.

Williams '433 does not specifically teach an insulator construct as described in claim 1 of the present application. In fact, the only point of commonality between Williams '433 and the present application is the use of the *barnase* gene as the lethal gene.

For all of the above reasons, Applicants respectfully submit that Williams '433 fails to anticipate the present invention. Withdrawal of the instant rejection is therefore respectfully requested.

The Examiner also rejects claims 1-4, 7-9, and 13-14 under 35 U.S.C. § 102(b) for allegedly being anticipated by Chang '042 (USP 5,610,042). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Chang '042 refers to a method for transformation of wheat plants by microprojectile bombardment. The male-sterility gene consists of (i) male-sterility DNA encoding a ribonuclease *(barnase)* under the control of a sterility promoter (TA29) and (ii) co-regulating gene consisting of a co-regulating DNA encoding a ribonuclease inhibitor *(barstar)* under the control of a co-regulating promoter (CaMV35S, Pnos). The two transcriptional units are placed in tandem one after the other and are not

separated by any defined insulator sequence as claimed in claims of the present application.

According to this strategy, the cytotoxic effect of barnase due to its leaky expression in tissues other than the tapetum is negated by the production of a one-to-one complex with barstar and this facilitates the development of male sterile plants.

The strategy will be effective only in cases where the lethal gene is always a ribonuclease encoding barnase and the inhibitor is barstar. It cannot be applied if any other lethal gene is used in place of *barnase*, if its inhibitor is not known.

On the other hand, the invention of the present application proposes a strategy whereby the leaky expression of any lethal gene can be negated by the use of appropriate insulators and separation of transcriptional units by the insulator sequence. Prior knowledge of inhibitors of lethal genes is not necessary in order to prevent leaky expression of the lethal gene. The function of the insulator sequence is to prevent the leaky expression of any lethal gene.

The 23 nucleotide long DNA sequence being referred to as an "insulator" by the Examiner on page 13 is an undefined region (with no specific defined function.) Such short stretches of DNA normally get introduced during conventional cloning procedures. They do not perform any function and are

not functional equivalents either of the insulator used in the present invention.

In addition, the sequence disclosed by Chang '042 is a very small sequence of DNA (~23 nucleotides). Consequently, the 23 nucleotide long sequence by itself cannot function as an insulator in the construct of the present invention to address the problem of leaky expression of a lethal gene. One cannot produce transgenic lines in rice, maize or *Brassica napus* by using the 23 nucleotide sequence as an insulator between the two transcription units as claimed in the present invention.

For all of the above reasons, Applicants respectfully submit that Chang '042 fails to anticipate the present invention. Withdrawal of the instant rejection is therefore respectfully requested.

***Rejection under 35 U.S.C. § 103***

The Examiner rejects claims 1-14, 16-21, and 24-28 under 35 U.S.C. § 103(a) for allegedly being unpatentable over Williams '433 (*supra*) in view of Mariani '041 (USP 5,689,041). The Examiner rejects claims 15 and 22-23 under 35 U.S.C. § 103(a) for allegedly being unpatentable over Williams '433 (*supra*) in view of Mariani '041 (supra) and further in view of Mathews et al (*Plant Science* 72:245 (1990)). Applicants respectfully traverse.

Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Williams '433 is directed towards development of homozygous male sterile lines and their corresponding maintainers. The 1200 base-pair sequence referred to by the Examiner as an 'insulator' is in fact, a functional transcription unit producing barstar protein as described in the foregoing paragraphs.

Sequence ID No. 3 is a plasmid containing transcription units for the *bar* and *barnase* genes. Sequence ID No. 17 gives the sequence of a HindIII fragment containing transcription units for the *barstar*, *bar* and *barnase* genes under different constitutive and tapetum-specific promoters wherein the *barstar* transcription unit is placed between the *bar* and *barnase* transcription units.

All these sequences encode some functional protein, whereas the insulator sequence of the present invention do not. Therefore, these sequences cannot be compared to the insulator sequence of the present invention.

Mariani '041 is directed to the restoration of male or female fertility in transgenic plants by neutralizing the effects of a cytotoxic gene (for example, *barnase*) using a specific inhibitor (for example, *barstar*) for the purpose of hybrid seed production in crop plants. In the absence of such neutralizing sequences, expression of the cytotoxic gene would

cause disruption of metabolic activities in certain plant cells leading to male or female sterility in such plants.

Mariani '041 also is directed to the production of male sterile plants using a suitable sterility inducing DNA (*barnase* or *RnaseT1*) driven by an appropriate tissue-specific promoter. The male sterility DNA is transformed into a plant that contains the restorer DNA for female fertility (in homozygous condition) as a part of a larger scheme for production of hybrid seeds in crop plants.

However, nowhere in the strategy is the deployment of the *barnase* gene in the absence of background expression of the barstar protein (either by constitutive promoters, for example 35S, pNos, pOcs; or by tissue-specific promoters, for example TA29) disclosed.

The present application primarily describes a method to protect tissue-specific regulation of lethal gene expression from enhancing effects of strong constitutive promoters used to drive a selectable marker gene in the same construct so as to enhance recovery of agronomically viable transgenic plants expressing the lethal gene in desired tissues. The described strategy could be used for example, for high frequency production of agronomically viable male sterile transgenic plants by protecting tissue-specific expression of a cytotoxic gene (for example, barnase).

Further, in constructs described by Mariani '041, the marker genes (for example *sfr*, *npt*, *hph*) have always been used with weak promoters (for example pssu, pNos) which might necessitate the use of different marker genes for *in vitro* and field selection. In contrast, the strategy described in the present application negates the requirement of an inhibitor protein in the background of transgenic plants containing the lethal gene and also enables the use of a single selectable marker gene for *in vitro* as well as field level selection thereby eliminating the need for redundant transgenes in crop plants.

***Why the cited art does not render the invention obvious***

The first of the cited art is Williams '433, which teaches the development of homozygous male sterile lines and their corresponding maintainer lines to address the issue of removal of male fertile plants from field conditions in hybrid seed production strategies. The 1200 base-pair sequence referred to by the Examiner as an 'insulator' is in fact, a functional transcriptional unit producing barstar protein as described in the foregoing paragraphs.

Mariani '041 teaches development of restorer lines using the barstar gene to inhibit *barnase* activity. Mariani '041 also describes development of male sterile *barnase* lines by expressing the *barnase* gene in a plant that contains the

restorer DNA for male fertility (in homozygous condition) as a part of a larger scheme for production of hybrid seeds in crop plants.

Mathews et al relates to a method for Agrobacterium-mediated transformation of *Brassica juncea*, which is a conventional method.

The hypothetical combination of Williams '433, Mariani '041 and Mathews et al to produce male-sterile plants using the *barnase* gene would work only in situations where the inhibitor protein of *barnase* (barstar) is expressed in the background. These approaches would not be successful for all lethal genes particularly in situations where prior knowledge of an inhibitor is not available. In contrast, the strategy described in the present invention has been shown to be effective in producing male-sterile plants even in the absence of inhibitor proteins. The inventive strategy is based on a totally different approach which completely prevents leaky expression of the lethal gene rather than using its inhibitor protein to negate its lethal effects following expression. This approach is therefore universally applicable for all lethal genes irrespective of whether functional inhibitors for the same are available.

Further, none of the cited art references teach how the problem of leaky expression of a lethal gene is to be addressed in the absence of any known inhibitors. The

identification of this problem and solution can be found in the present application.

For obviousness, the cited references must directly or indirectly suggest or teach the principles on which the claimed invention is based. The suggestion as well as the likelihood of success must both be found in the prior art and not in the patent application at hand.

A person of ordinary skill when charged with the knowledge of Williams '433 and Mariani '041 would not be able to arrive at the present invention because the skilled artisan would not know that the leaky expression of any lethal gene can be prevented by distancing it from the adjacent unit (containing a strong constitutive promoter) using an insulator sequence which does not produce any functional RNA or protein. This teaching is completely absent in the cited art.

The present Inventors have worked on the methods of the prior art and thereafter, developed a novel construct containing a lethal gene and a marker gene, both separated by an insulator sequence which does not produce any RNA or protein.

The question, therefore, is whether (a) the selection of the insulator sequence from the thousands of sequences available, (b) the combination thereof with a lethal gene, and (c) the arrangement of the sequences in the construct, such that the leaky expression of the lethal gene is negated, as

disclosed in the present invention, is obvious over the prior art, given that the cited art does not identify use of an insulator sequence which does not encode any functional RNA or protein to prevent leaky expression of a lethal gene in the absence of its corresponding inhibitor.

The novelty of the invention resides in Applicants' intelligent appreciation of the problem which faced with the art prior to their work, their intelligent selection of the insulator sequence to solve this problem having in mind both desirable and undesirable effects, and finally bringing together a particular combination of such materials.

Once applicants have taught the art what the problem was and have disclosed their selection and combination of materials to solve the problem - then, but not until then, the invention may become a matter 'obvious' or 'routine work'.

The strategies in the prior art may be "obvious to try" but they do not indicate how to solve the problem of leaky expression of lethal genes in the absence of corresponding inhibitors. When the cited art does not teach the insulator sequence at all, no good teaching can be gleaned from the citations by a person skilled in the art.

Therefore, contrary to the position taken by the Examiner, the cited references do not render the invention obvious. Withdrawal of the instant rejection is requested.

***Conclusion***

In view of the above amendments and comments, Applicants respectfully submit that the claims are in condition for allowance. A Notice to such effect is earnestly solicited.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicants hereby petition for an extension of two (2) months to April 15, 2002, in which to file a reply to the Office Action. The required fee of $400.00 is enclosed herewith.

If the Examiner has any questions concerning this application, he is requested to contact Kristi L. Rupert, Ph.D. (45,702) at (703) 205-8000 in the Washington, D.C. area.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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RCS/KLR:gml
Attachment

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

**IN THE CLAIMS**:

The claims have been amended as follows:

1. (Amended) An [I]insulator construct for controlling leaky expression of a lethal gene from enhancing functions of a strong constitutive promoter present in the said [I]insulator construct following integration into the genome of a plant, said [I]insulator construct comprising:

i) first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and fused to a suitable transcription termination signal, [including] comprising a polyadenylation signal,

ii) second transcription unit comprising a selectable marker gene [DNA] under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, [including] comprising a polyadenylation signal, and

(iii) an [I]insulator sequence placed between the first and second transcription units so as to isolate the first transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit.

3. (Amended) The construct as claimed in claim 1 wherein the lethal gene is selected from the group comprising *barnase* gene, *RnaseTI* gene, *binase* gene, *rolB* gene, *rolC* gene and diphtheria [Diphtheria] toxin A [chain-coding] gene.

4. (Amended) The construct as claimed in claim 1 wherein the [preferred] lethal gene *is barnase* gene.

6. (Amended) The construct as claimed in claim 1 wherein the [preferred] tissue specific promoter is TA29.

7. (Amended) The construct as claimed in claim 1 wherein the marker gene [of the second transcription unit] is selected from the group of herbicide resistance-conferring genes consisting of [comprising] *bar* gene, *ALS* gene, and *tfdA* gene, or from the group of antibiotic resistance-conferring genes consisting of [comprising] *nptII* gene, *hpt* gene and *aadA* gene.

8. (Amended) The construct as claimed in claim 1 wherein the [preferred] marker gene is *bar* gene.

9. (Amended) The construct as claimed in claim 1 wherein the strong constitutive promoter [for expression of the *bar* gene] is CaMV35S promoter.

10. (Amended) The construct as claimed in claim 1 wherein the [I]insulator sequence of about 5kb comprises coding sequences of *topoisomerase* gene from pea and *acetolactate synthase* gene from *Arabidopsis* [sequence derived from genomic DNA of a plant].

11. (Amended) The construct as claimed in claim [1] 10 wherein the [I]insulator sequence has [a] the following properties: [length of at least 2kb.]

(a) does not encode any functional or regulatory components or possess any regulatory or enhancer elements or sequences that may influence the expression of neighboring genes;

(b) GC content of the sequence is in consonance with transcriptionally active regions of the host genome;

(c) does not produce any functional RNA or protein; and

(i) does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.

12. (Amended) The construct as claimed in claim [1] 10 wherein the [preferred] length of the [I]insulator sequence is about 5kb.

13. (Amended) A male sterile transgenic plant [and parts] or seeds thereof which contain in their nuclear genome the construct of claim 1.

15. (Amended) The [preferred] plant [as claimed in] of claim 13 wherein the plant is [is a dicotyledonous plant] *Brassica juncea*.

16. (Amended) A method to obtain male-sterile plants, said method comprising the steps of:

i) transforming the nuclear genome of plant cells with a foreign DNA comprising:

a) a first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and

fused to a suitable transcription termination signal, [including] comprising a polyadenylation signal,

b) a second transcriptional unit comprising a selectable marker DNA under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, [including] comprising a polyadenylation signal,

c) an [I]insulator [DNA] sequence [derived from plant genomic sequences] placed between the first and second transcription units, so as to isolate the first transcription unit from enhancing influences of the [constitutive] constitutively expressing promoter in the second transcription unit ;[.]

ii) regenerating plants from said transformed plant cells,

iii) [identification] identifying [of] male sterile transgenic plants by [morphological observations] the absence of pollen production and by their failure to set seed on selfing,

iv) obtaining, at a high frequency, male sterile plants with normal vegetative morphology and normal female fertility,

v) identifying single copy male sterile lines by Southern hybridization,

vi) back-crossing male sterile plants with untransformed parent to obtain T1 seeds,

vii) obtaining male sterile plants with normal T1 seed germination frequencies

viii) obtaining normal segregation ratio of marker gene among T1 progeny of single copy male sterile plants identified, and

ix) obtaining stable transfer of male sterile phenotype among all T1 plants exhibiting marker resistance.

17. (Amended) A method as claimed in claim 16 wherein the [preferred] lethal gene is *barnase* gene.

18. (Amended) A method as claimed in claim 16 wherein the [preferred] tissue specific promoter is TA29.

19. (Amended) A method as claimed in claim 16 wherein the [preferred] marker gene is *bar* gene.

20. (Amended) A method as claimed in claim 16 wherein the [preferred] constitutive promoter is CaMV35S promoter.

21. (Amended) A method as claimed in claim 16 wherein the [preferred] length of the [I]insulator sequence is about 5kb.

22. (Amended) A method as claimed in claim 16 wherein the plant used for transformation is [the dicotyledonous plant] *Brassica juncea*.

23. (Amended) A method as claimed in claim 16 wherein male sterile lines in *Brassica juncea* are generated [preferably] by *Agrobacterium*-mediated transformation using disarmed Ti plasmid.

24. (Amended) A method as claimed in claim 16 wherein the male sterile transgenic plants are backcrossed [to the] with untransformed parent to obtain T1 seeds [generate progeny and tested for female fertility as evidenced by their ability to set seed in crosses].

25. (Amended) A method as claimed in claim 16 wherein [the] single copy male sterile plants are [analyzed] identified by Southern hybridization [to identify transgenic plants containing a single copy of the T-DNA insert].

26. (Amended) A method as claimed in claim 16 wherein T1 seeds [obtained from backcrossing the above male sterile plants] of claim 25 are tested for their viability as evidenced by their ability to germinate on non-selective media.

27. (Amended) A method as claimed in claim 16 wherein germinated T1 seedlings obtained from backcrossed seeds were tested for segregation of the marker gene by transferring them on selective media.

28. (Amended) A method as claimed in claim 16 wherein the T1 [plants] progeny [obtained from selected backcrossed progeny] were transferred to field conditions and tested for stable inheritance of the male sterile phenotype.

Claims 29-31 have been added.